Environmental Health Perspectives
Vol. 37, pp. 79-84, 1981

Maize Pollen Test Systems to Detect Nondisjunction

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Three pollen test systems to detect the induction of nondisjuction in maize are actively being explored. (1) Each member of a tetrad of haploid microspores produced by meiosis contains a single chromosome 6 which carries the only nucleolar organizing region in the maize genome. Thus, each member of a normal tetrad contains one nucleolus. If nondisjunction took place at the first or second meiotic division, 2:2:0:0 or 2:1:1:0 tetrads would be produced respectively. (2) If a male parent carrying a dominant endosperm marker is crossed by a female carrying a recessive allele of this gene, all normal kernels would be heterozygous and would express the dominant phenotype in their endosperm. If nondisjunction of the chromosome carrying this gene took place at the second microspore division and the nullisomic and disomic sperm fertilized the polar nuclei and egg of a given embryo sac respectively, an exceptional kernel is produced which would express the recessive endosperm phenotype and contain a trisomic embryo. (3) Complementing null mutations of genes expressed in individual pollen grains can be utilized to detect nondisjunction. Normal haploid pollen grains from plants heterozygous for two complementing null alleles of a locus would each express the recessive phenotype. If nondisjunction took place during either meiotic division, disomic pollen grains containing both alleles could be produced expressing the dominant phenotype due to complementation. We are exploring this test system utilizing appropriate alcohol dehydrogenase mutants.

About 15% of the recognized human pregnancies abort spontaneously, and of these, about half are chromosomally abnormal. Nearly 70% of these chromosomally abnormal individuals are trisomic or monosomic. Although most aneuploids are eliminated by abortion during pregnancy, about 6% of the babies who die perinatally (2%) are chromosomally abnormal (the majority are trisomics, especially for group E chromosomes). Of the survivors, about 0.36% are aneuploid (*1*). In addition to the suffering endured by parents who lose a child prenatally or perinatally or who bear a surviving child who is abnormal due to aneuploidy, many of the surviving aneuploid children must be institutionalized throughout their lifetimes at great expense to society. Clearly, the possibility that some environmental agents might be capable of bringing about an increase in the frequency of aneuploidy is a real cause for concern.

Nondisjunction is the misdivision of a cell such that one daughter cell receives an extra chromosome and the other daughter cell lacks a copy of that chromosome. Test systems currently in use to detect the induction of aneuploidy by environmental mutagens were discussed exhaustively at a recent workshop sponsored by the National Institutes of Environmental Health Sciences (*2*). Although efficient and unequivocal test systems for nondisjunction are available utilizing fungi as test organisms (*3–6*), test systems employing higher eukaryotic plants and animals are cumbersome, inefficient, expensive, or the event indicative of nondisjunction in the test system is equivocal. Because of the differences known to exist between lower and higher eukaryotic organisms in nucleosomes, relative amounts of repetitive and nonrepetitive DNA, cell division mechanisms (many fungi carry out their mitotic division without dissolution of their nuclear membranes), and others, it is unwise to make extrapolations from lower eukaryotes to higher eukaryotes. It is also thoroughly documented that specific gene mutagens exert profoundly different effects on different organisms. Clearly it is important to examine the effects of specific mutagens on a wide spectrum of organisms.

For these reasons, we are developing test systems to detect the occurrence of nondisjunction

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utilizing *Zea mays* (maize), the higher eukaryotic plant with the most distinguished genetic and cytogenetic history. Three of these test systems are pollen test systems, and we are intensively exploring each of these test systems. They are described below.

Test System One

This test system is an ordered full tetrad analysis and would unequivocally detect nondisjunction at either meiotic division is maize or any other angiosperm which contains a single nucleolar organizing region in its haploid genome. The nucleolar organizing region (NOR) is that portion of the genome responsible for the formation of the nucleolus (*7*). In maize, chromosome 6 bears the only NOR in the haploid genome.

In a normal meiotic division, the two homologous chromosome 6's pair, pass to opposite poles at anaphase I, and divide at anaphase II to produce a tetrad of haploid microspores as diagrammed in Figure 1. The four members of each tetrad are in a single plane in maize and remain associated with each other for some time after meiosis is completed. Because each member of the tetrad contains a single chromosome 6 which bears the NOR, each member of the tetrad contains one nucleolus. A normal tetrad is shown in Figure 2*a*.

When nondisjunction of chromosome 6 takes place at the first meiotic division, both chromosome 6's pass to the same pole at anaphase I and divide at anaphase II to produce an exceptional tetrad with two chromosome 6's in two adjacent cells and none in the other two cells (Fig. 1). The two cells lacking NORs do not form a nucleolus, but numerous bodies resembling small nucleoli (nucleolar blebs) are present in these cells (*7*). Such tetrads are designated as 2:2:0:0 tetrads and can be easily recognized in propiocarmine squash preparations (Fig. 2*b*).

When nondisjunction of chromosome 6 takes place at the second meiotic division, a tetrad is produced which contains two chromosome 6's in one cell, one in two, and none in one (Fig. 1). Such a tetrad is designated as a 2:1:1:0 tetrad (Fig. 2*c*). Clearly, a 2:2:0:0 tetrad is the unequivocal result of nondisjuction of chromosome 6 at the first meiotic division and a 2:1:1:0 tetrad is the unequivocal result of nondisjunction of chromosome 6 at the second meiotic division; thus, nondisjuction of chromosome 6 at either meiotic division can be unequivocally identified utilizing this protocol.

Another exceptional event which could produce

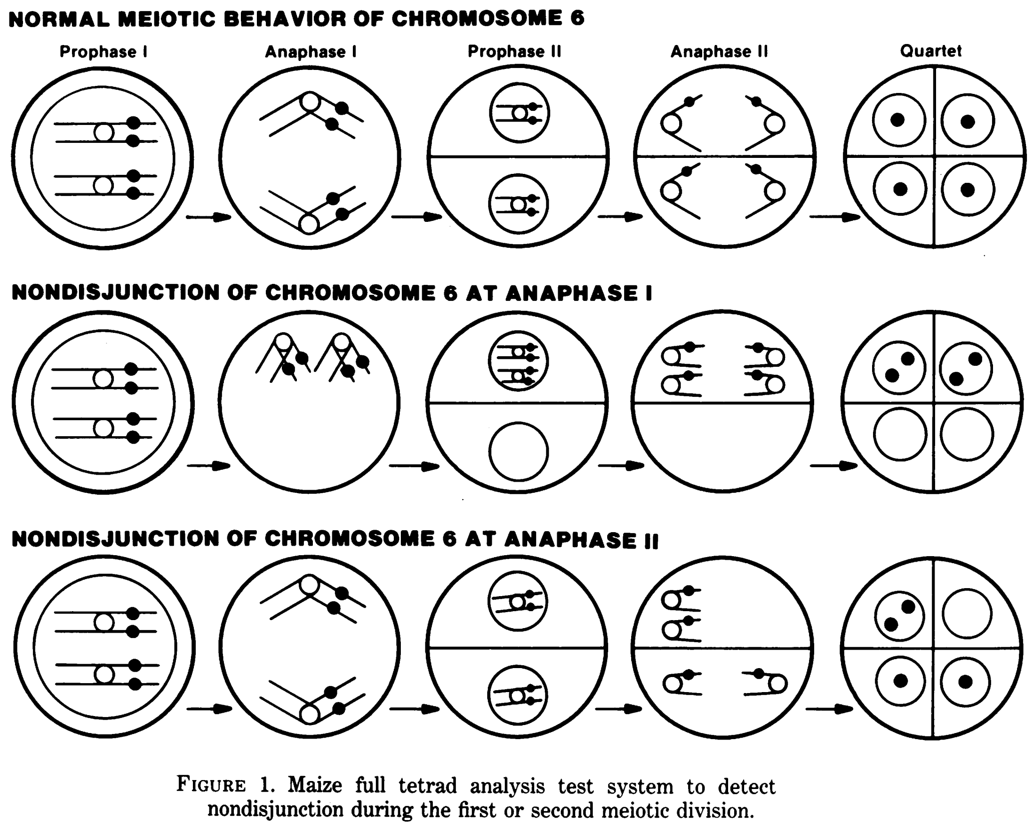
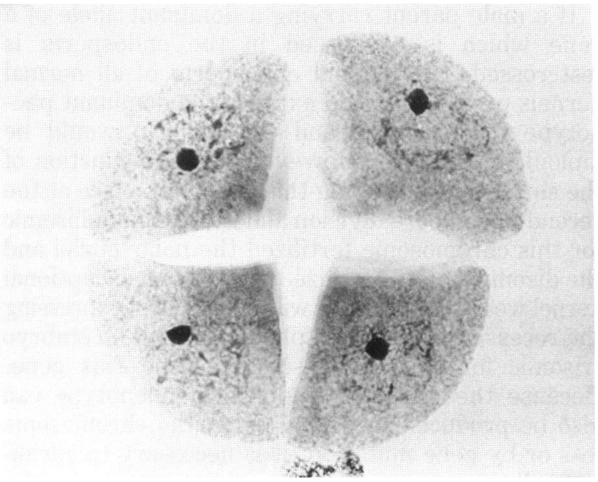
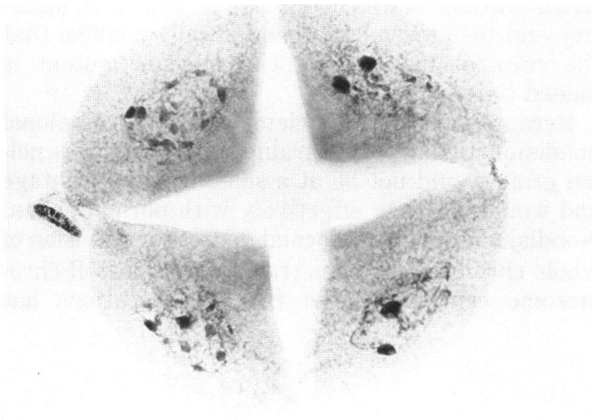


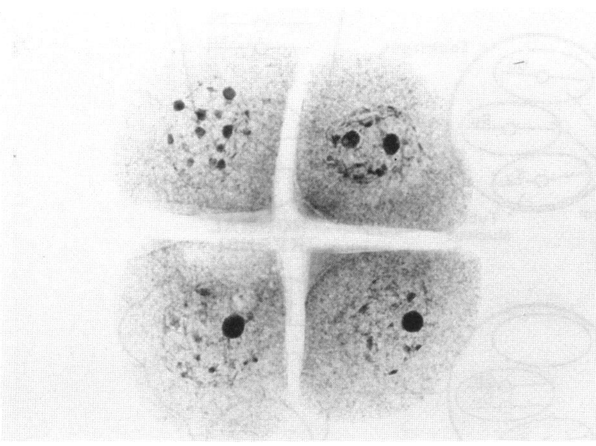
FIGURE 1. Maize full tetrad analysis test system to detect nondisjunction during the first or second meiotic division.



(*a*) 1:1:1:1



(*b*) 2:2:0:0



(*c*) 2:1:1:0

FIGURE 2. Maize tetrads.

these same quartet types would be the formation of a reciprocal translocation with one breakpoint between the NOR and the centromere on chromosome 6. This type of event would be extremely rare because the NOR is located very close to the centromere on chromosome 6 in maize.

Unfortunately, the two nucleoli in a cell containing two NORs sometimes fuse into a single nucleolus; thus, 2:1:0:0, 1:1:0:0, and 1:1:1:0 tetrad would also be produced as a result of nondisjunction of chromosome 6. These exceptional tetrads would also be produced as a result of loss of chromosome 6 during meiosis; thus, these exceptional tetrad types could not be utilized to determine nondisjunctional frequencies.

A single individual can prepare slides and classify approximately 500–1,000 quartets per hour; thus, a reasonably large number of quartets can be classified utilizing this protocol. No exceptional tetrads were observed among 40,000 examined from untreated plants; thus, spontaneous nondisjunction of chromosome 6 rarely occurs during meiosis in maize.

An important advantage of this protocol is that no special stocks are used. One could analyze field corn growing in farmer's fields to determine if agricultural chemicals used or environmental pollutants present have increased nondisjunctional frequencies in these plants. Also, it is not necessary to grow the plants to sexual maturity to utilize this protocol. Samples are taken from typical plants approximately 45 days after planting under field conditions. Other lines, such as Early Early Synthetic, are available which reach this stage in approximately half this time. Several thousand quartets can be analyzed from each meiotic sample taken from a single plant.

It is important to note that both the hypoloid and hyperploid products of nondisjunction at either meiotic division are recovered in a single tetrad of microspores utilizing this protocol. If nondisjunction of chromosome 6 took place prior to meiosis, it would result in the production of trisomic and monosomic diploid clones which would produce 2:2:1:1 and 1:1:0:0 tetrads, respectively. These would not be classified as a positive result of meiotic nondisjunction. Thus, it can be determined with certainty that nondisjunction occurred during meiotic divisions.

I have recently explored the effect of the loss of other members of the haploid genome on the production of nucleoli at the quartet stage (*8*). I found that microspore cells nullisomic (totally lacking) for chromosomes 1, 2, 4, 7, 8, 9, or 10 still produce nucleoli which appear normal in propiocarmine squash preparations; thus, no factors are located on these chromosomes which also are necessary for nucleolar formation in microspores at the quartet stage. Cells nullisomic for chromosomes 3 and 5 were not explored because they are not yet available. Thus, nondisjunction of these other chromosomes would not result in the production of altered tetrad configurations.

If one wanted to study nondisjunction of other centromeres in the maize genome, one would simply utilize plants which are homozygous for a reciprocal translocation where one breakpoint was between the centromere and the NOR of chromosome 6 and the other breakpoint was on another chromosome in the genome. In this way, the nucleolar organizing region is transposed onto another centromere and nondisjunction of that centromere could readily be analyzed. Translocations of this type involving several different chromosomes are available in maize (*9*).

Test System Two

This test system is designed to detect mitotic nondisjunction at the second microspore division of pollen formation. After meiosis is completed in maize, each haploid microspore nucleus divides into a tube nucleus and a generative nucleus, and the generative nucleus undergoes a second mitotic division to produce two sperm nuclei as diagrammed in Figure 3. In maize, both microspore divisions take place before the pollen is shed; thus, maize pollen is trinucleate.

If a male parent carrying a dominant allele of a gene which is expressed in the endosperm is testcrossed, the triploid endosperm of all normal kernels produced would express the dominant phenotype of this gene and the embryo would be diploid (see Fig. 3). However, if nondisjunction of the chromosome bearing this gene took place at the second microspore division and the sperm nullisomic for this chromosome fertilized the polar nuclei and the disomic sperm fertilized the egg, an exceptional kernel would be produced with endosperm expressing the recessive endosperm phenotype and an embryo trisomic for the chromosome bearing this gene. Because the recessive endosperm phenotype can also be produced by whole or partial chromosome loss or by gene mutation, it is necessary to germinate all exceptional kernels and determine somatic chromosome numbers from root tips. These exceptional trisomic plants could also be grown to maturity and testcrossed to unequivocally confirm that the chromosome bearing the marker mutant is indeed trisomic.

Because the tube nucleus of this exceptional nondisjunctional pollen grain is balanced, this pollen grain would not be at a selective disadvantage and would compete effectively with normal pollen. Nondisjunction at the second microspore division of whole chromosome arms translocated onto B chromosome centromeres in B-A translocations has

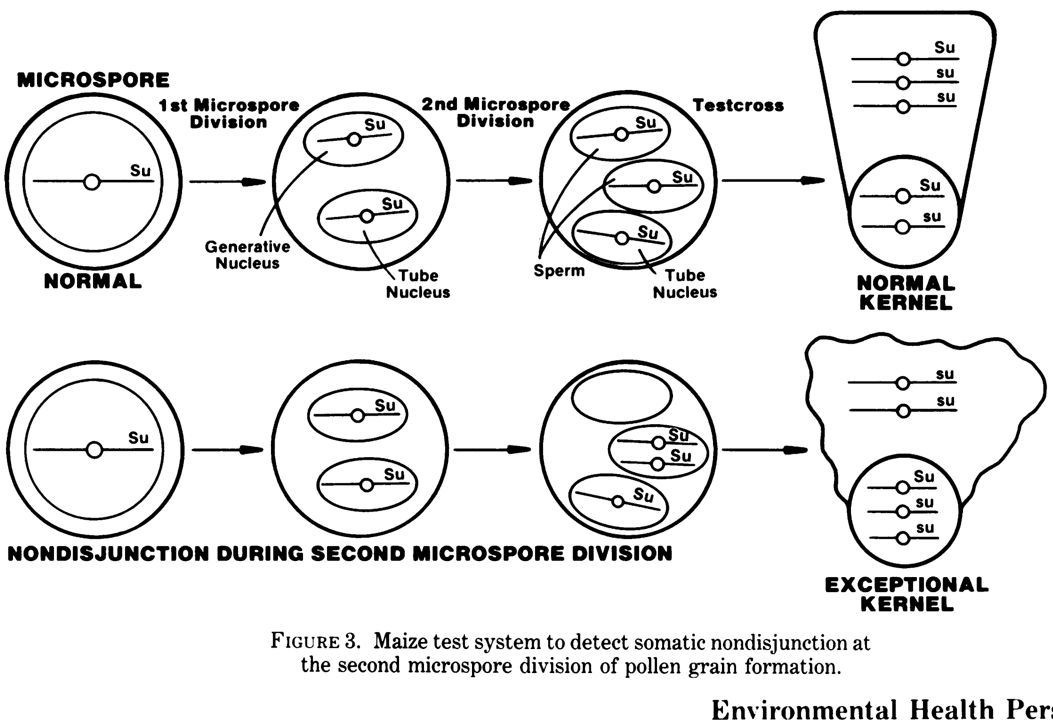


FIGURE 3. Maize test system to detect somatic nondisjunction at the second microspore division of pollen grain formation.

been intensively studied by many investigators (*10*, *11*), and hypoploidy of whole chromosome arms in the triploid endosperm is of little if any consequence to the normal development of endosperm. For this reason, loss of an entire chromosome arm should have little, if any detrimental effect on the development of endosperm.

An important feature of this test system is that it is possible to simultaneously monitor nondisjunctive frequencies in several different chromosomes if a female tester parent is utilized which bears phenotypically distinguishable recessive endosperm mutations on several different chromosomes. In human abortuses, trisomies of different chromosomes occur at vastly different rates suggesting a differential rate of generation (*1*). Trisomy 16 is clearly the most common trisomy, accounting for 31.5% of all trisomies, while trisomies for chromosomes 1, 5, 6, 11, 12, 17, 19, and X added together account for only 2.1% of all trisomies. Most, if not all of the efficient test systems currently employed to determine nondisjunctional frequencies monitor nondisjunction of a single chromosome. It is important to determine if different chromosomes undergo different rates of spontaneous or induced nondisjunction.

This test system also employs no special stocks as male parents, and one could cross field corn growing in agricultural fields by appropriate tester stocks to determine if specific environmental factors (agricultural chemicals or other pollutants) increased the frequency of nondisjunction. Unfortunately, this test system requires that the plants be grown to sexual maturity and utilized in crosses to produce progeny. Thus, the time interval required for this test system would be 4–5 months. It is important to note this test system also identifies both the hypoploid and hyperploid daughter cells resulting from nondisjunction at a specific division, and with this test system there can be absolutely no question that nondisjunction has taken place.

Test System Three

A typical maize plant produces about 2×10^7 pollen grains; therefore, a test system capable of detecting nondisjunction directly in individual pollen grains would be an extremely powerful system. One locus expressed in the pollen is the alcohol dehydrogenase-1 gene *Adh1* gene; ADH enzyme, EC 1.1.1.1). Freeling (*12*) developed cytochemical staining procedures for maize pollen to distinguish between ADH– pollen grains, which lack detectable levels of alcohol dehydrogenase and stain yellow and translucent, and ADH+ pollen grains, which contain alcohol dehydrogenase activity and stain blue and opaque. Schwartz has recovered numerous *Adh1*-deficient alleles of this locus (*11*). Freeling has generously provided me with several of these alleles. Many of these *Adh1*-deficient mutants have been characterized and some have been found to be CRM +. Specific combinations of these deficient CRM + alleles (such as W/C^{m}) have been shown to complement each other. These complementing *Adh1*-deficient mutants which are expressed in the pollen can be utilized in a powerful nondisjunction test system as described below:

Plants heterozygous for two complementing *Adh1*-deficient mutants would be grown to anthesis (the time of pollen shed) and pollen from such plants would be collected and stained for the ADH reaction utilizing the protocol developed by Freeling (*12*). Normal haploid pollen grains bearing either parental allele would give the ADH– reaction and would be yellow and clear. If nondisjunction took place at either meiotic division and both alleles were incorporated into a disomic pollen grain, complementation would take place to produce an ADH+ pollen grain, and this pollen grain would stain blue and opaque. Because the *Adh1* gene is over 50 map units from the centromere on chromosome 1, half of the anaphase I chromosomes of a heterozygous plant would carry the two different alleles on the two chromatids attached to a given centromere. Thus, 75% of the exceptional disomic pollen grains generated from nondisjunction at anaphase I would be heteroallelic and 25% would be homoallelic, and 75% of the disomic pollen would be ADH +. The other product of a nondisjunctive event at anaphase I is a nullisomic pollen grain which would abort; however, because a low frequency of pollen abortion takes place in normal plants, this event could not be identified. If nondisjunctions of chromosome 1 took place at the second meiotic division to produce a disomic pollen grain, approximately half of these exceptional pollen grains would also give a positive ADH reaction because approximately half of the time the two chromatids of an anaphase I chromosome would be heteroallelic. Thus, 75% and 50% of the disomic pollen produced by nondisjunction at the first or second meiotic divisions respectively would give a positive ADH reaction.

Three other types of events can also produce ADH+ pollen grains, namely, "revertants," intragenic recombination, and formation of diploid pollen grains. If a specific agent produced an increased frequency of ADH+ pollen grains, additional tests would be necessary to determine that the increased frequency was not generated by one of these phenomena. First, the frequency of ADH+ "revertants" in each of the two homoallelic plant types would need to be determined after each treatment. These ADH+

"revertants" might be the result of true backmutation, conversion, suppression by any mechanism, activation of a repressed *Adh1*, an overproducer, or the like. The mean frequency of "revertants" found in the two homoallelic combinations would need to be subtracted in each case. Second, it would be necessary to determine if the treatment has resulted in an increased frequency of intragenic recombination between the two mutational sites, and this was responsible for the increase. For this purpose it would be necessary to determine if the experimental treatment caused an increased frequency of ADH+ pollen to be found in plants heteroallelic for two *Adh* deficient alleles which do not complement. If a specific agent greatly increased intragenic recombination, this would be of great interest. Alternatively, two *Adh1*-deficient complementing mutations could be used in which intragenic recombination between the two alleles occurs at an undetectable level. Several combinations of this type are available (*13*). Third, it would be necessary to determine which ADH+ pollen grains are heteroallelic diploid pollen grains. These can be formed from restitution nuclei, or in tetraploid sectors. They can be readily distinguished because diploid pollen grains are larger than haploid pollen grains.

Clearly, this third test system would enable the efficient analysis of agents which induce a low frequency of nondisjunction. An important advantage of this test system is that it is not necessary to grow plants to maturity. Freeling estimates (*14*) one person can collect from 50 plants in one hour; the total freezing-staining process could consume less than 3 man-hours; the stained pollen may be stored indefinitely. Counting is done in a laboratory with each estimate taking 30–45 min. He has been able to resolve reversions frequencies at 10^7 utilizing this locus.

Three test systems utilizing maize pollen have been described. I might point out that benomyl, the active ingredient in Benalte, has been shown to induce nondisjunction in fungal test systems (*15*, *16*). This same chemical is utilized as a fungicide on soybeans, and a large portion of the soybeans raised for seed production in the United States are treated with this chemical. If this chemical increases the nondisjunction rate in soybeans as it does in fungal test systems, this might be extremely detrimental to the soybean germplasm and to the individuals who consume soybean products. No good plant test systems currently exist where this could be determined.

This work was supported in part by DOE Contract DE-AC02-76EV02121.A005.

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